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(71) Applicant *(for all designated States except US)*: PEPTECH(UK) LIMITED [GB/GB]; One St Paul's Churchyard, London EC4M 8SH (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: IVANOV, Vadim Tikhonovich [RU/RU]; M M Shemyakin and Yu A Ovchinnikov, Institute of Bioorganic Chemistry Russian Academy of Sciences, 16/10 Muklukho-Maklaya Street, Moscow, 117871 (RU). MIKHALEVA, Inessa Ivanovna [RU/RU]; M M Shemyakin and Yu A Ovchinnikov, Institute of Bioorganic Chemistry Russian Academy of Sciences, 16/10 Muklukho-Maklaya Street, Moscow, 117871 (RU). ONOPRIENKO, Ludmilla Viktorovna [RU/RU]; M M Shemyakin and Yu A Ovchinnikov, Institute of Bioorganic Chemistry, Russian Academy of Sciences, 16/10 Muklukho-Maklaya Street, Moscow, 117871 (RU). VOITENKOV, Boris Olegovich [RU/RU]; N N Petrov Research Institute of Oncology of the Russian Federation, Ministry of Health, 68 Leningradskaya Street, Pesochny-2, Sanot Petersburg, 189645 (RU). OKULOV, Valery Borisovich [RU/RU]; N N Petrov Research Institute of Oncology of the Russian Federation, Ministry of Health, 68 Leningradskaya Street, Sanot Petersburg, 189645 (RU).

(74) Agents: ROBERTS, Alison, Christine et al.; Kilburn & Strode, 30 John Street, London WC1N 2DD (GB).

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(57) Abstract

Peptides derived from IL-2 and containing amino acids from the region extending from residues 58 to 72 of IL-2 are useful in the treatment of IL-2 mediated disease, although they do not contain the recognised binding region of IL-2.

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PEPTIDES DERIVED FROM HUMAN INTERLEUKIN-2 FOR USE IN MEDICINE

The present invention relates to interleukin-2 (IL-2) derived peptides that are useful in the treatment of inflammation, in tissue reparation and in the treatment of cancer in a broad sense.

IL-2 is a 15.5 kiloDalton glycoprotein synthesised by lymphocytes following their activation by mitogens or antigens. In addition to being an essential growth factor for T lymphocytes, IL-2 has a range of other biological functions. These include the induction of antigen specific cytotoxic T-lymphocytes, and the activation of "natural killer cells" or lymphokine activated killer cells. These and other activities have demonstrated the importance of IL-2 in a number of physiological processes involving the immune system, particularly involving defence against tumours and activation of cells at sites of infection and defence. Moreover, associations have also been reported between IL-2 and diseases such as lupus erythematosus and primary immunodeficiencies.

However, IL-2 is a toxic therapeutic agent (Vial, T & Descotes, J; *Drug Safety*, 7, 417-433 (1992)) causing the manifestation of serious side effects, including cancer of the kidney and therefore it is desirable to develop agents which mimic aspects of IL-2 activity but which do not have the harmful side effects of IL-2. In particular, it would be advantageous to develop agents which regulate IL-2 activity or which can modulate the production of other cytokines may have useful therapeutic application in immunoregulation, inflammation , tissue repair or cancer.

A conventional strategy to develop such agents would be to isolate a part of the IL-2 molecule which is able to reproduce some of its beneficial activities, whilst avoiding its unwanted side effects. The obvious choice for such agents would be the part of the molecule suspected of interacting with the receptor. IL-2 derived peptides that appear to interact with the receptor have been described (Zav'yalov *et al*, *Immunology Letters* 31, 285-288, 1992), and some of these compete with binding of full-sized IL-2 to its receptor. The regions claimed to antagonise IL-2 receptor binding were in the region of amino acid residues 27-35. Furthermore, this region was previously suggested to be involved in receptor binding from X-ray analysis of IL-2 structure (Zurawski and Zurawski, *EMBO J.* 8, 2583-2590, 1989).

However, the present inventors have surprisingly found a completely different region of the IL-2 molecule which appears to be implicated in its biological activity and, in particular its tissue reparation, anti-inflammatory and anti-cancer properties.

Therefore, in a first aspect of the present invention there is provided a peptide of not more than 50 amino acids in length and comprising a fragment of at least 5 amino acids of the region extending from amino acid residues 58 to 72 of IL-2 and having the sequence:

```
58  59  60  61  62  63  64  65  66  67  68  69  70  71  72
Cys-Leu-Glu-Glu-Glu-Leu-Lys-Pro-Leu-Glu-Glu-Val-Leu-Asn-Leu;
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a fragment substantially homologous thereto; or a derivative of such a fragment, for use in medicine.

These peptides have the desirable properties of IL-2 mentioned above whilst lacking the undesirable side effects of full length IL-2. The activity of the peptides is particularly surprising since the region from amino acids 58 to 72 of IL-2 is structurally distinct from the receptor binding region at residues 27 to 35.

Peptide 59-72 of IL-2 was previously described by Kuo and Robb (*J. Immunol.*, **137**, 1538-1543, 1986), who used it in an investigation of the IL-2 receptor binding site. These authors did not obtain evidence that this peptide was involved in binding to the receptor. They found that it did not cause stimulation of IL-2 dependent cell lines, and they did not demonstrate any biological activity for it.

Altman *et al*, (*Proc. Natl. Acad. Sci. USA*, **81**:, 176-2180, 1984) synthesised a peptide corresponding to amino acids 59 to 72 of IL-2 (referred to as 79 to 92 in that publication which used a different numbering system). This peptide was one of several used to raise antibodies to IL-2. No evidence was gained for a functional role of this part of the molecule.

In the present invention, the term "derivative" relates to a peptide having the amino and/or carboxy terminus blocked, for example by conversion of the carboxy terminus into an amide or ester derivative or to a peptide in which one or more of the amino acids is substituted or has the D configuration.

The term "substantially homologous" would be well understood by one skilled in the art who would easily be able to determine whether or not two sequences were

substantially homologous. However, in general, amino acid sequences can be described as substantially homologous when they have at least 40% homology although, for the purpose of the present invention, it is preferable for a sequence to have at least 50%, 60%, 70%, 80%, 90% or 95% homology (in increasing order of preference) to the amino acid sequence 58 to 72 of IL-2. In addition, the residues which are compared need not be in exactly the same positions in two sequences which are substantially homologous but rather, one of the sequences may have various inserted or deleted amino acid residues or regions with respect to the sequence with which it is compared.

The term "C_1-C_{20} alkyl" refers to a straight or branched chain alkyl group containing from one to 20 carbon atoms. Examples of such groups include methyl, ethyl, n-butyl, tertiary butyl, decyl, dodecyl and stearyl.

The term "C_2-C_{20} alkenyl" refers to a straight or branched chain hydrocarbon having at least one double bond. Examples include 1-propenyl and 1-hexenyl.

The region of the human IL-2 molecule, with the amino acid positions numbered from 58 to 72 is as follows:

58 59 60 61 62 63 64 65 66 67 68 69 70 71 72
Cys-Leu-Glu-Glu-Glu-Leu-Lys-Pro-Leu-Glu-Glu-Val-Leu-Asn-Leu

and the present invention relates to peptides comprising this region or a fragment of this region of at least five amino acids in length, peptides containing a region substantially homologous to the above region or a fragment of at least five amino acids of the above region

or derivatives of either of the above. The peptides may also be either linear or cyclic although linear forms are preferred.

Although the peptide may be up to 50 amino acids in length, it is preferred that it is somewhat shorter than this, for example no more than 40, 30 or even 20 amino acids long.

Derivatives of the peptides which may be used include the following.

The C-terminal carboxylic acid may be converted to a substituted or unsubstituted C_1-C_{20} alkyl amide, C_2-C_{20} alkenyl amide, aryl amide, C_1-C_{20} alkyl or C_2-C_{20} alkenyl ester, aryl ester, C_1-C_{20} alkyl or C_2-C_{20} alkenyl ether or aryl ether. Examples of such groups include phenyl ester, benzyl ether or thiophenylethyl ester but C_1-C_3 alkyl ethers and esters, and especially the methyl ester, are particularly suitable. Suitable substituents for alkyl, alkenyl and aryl groups include hydroxy, amino and halogen substituents.

Cysteine residues of the peptides of the invention may be substituted by C_1-C_{20} alkyl or C_1-C_{20} alkenyl amide or substituted amide, triphenylmethyl, a C_1-C_{20} alkyl or C_2-C_{20} alkenyl substituted carbamoyl group or a benzyl group.

The N-terminal amino acid of the peptide may be N-substituted with a group such a saturated or unsaturated aliphatic C_1-C_{20} acyl group or by an aromatic acyl group. Suitable substituents include

formyl, acetyl and propionyl groups.

The derivatisation of the N and C terminals of the peptide and the cysteine groups helps to confer resistance to enzymatic degradation.

Other suitable substituents include those in which one or more nitrogen atoms are replaced by oxygen atoms such that the amide bonds are replaced by ester bonds. In addition, hydroxy side chains may be derivatised to form C_1-C_{20} ester or ether groups and/or at least one amino acid may be substituted by a monomeric or polymeric carbohydrate or a derivative thereof *via* a hydroxy, amino or amido group of the amino acid.

In the case of cyclic peptides, it is preferred that at least one additional cysteine residue is present. In order to cyclise the peptide, an S-S bridge can then be formed between two cysteine residues.

The peptides can be substituted in other ways but it is generally preferred that the substitutions are conservative in nature. That is to say, that the substituents will resemble the groups they replace in size, polarity and acidity or basicity.

Substitutions in which the L-amino acids which occur in natural IL-2 are replaced by the equivalent D-amino acids are often particularly useful since they often increase the half life of the peptide *in vivo* and, in addition, they increase the resistance to enzymatic degradation.

Other derivatives which are useful in the present invention include multimers, for example, dimers,

trimers, tetramers or pentamers of the peptides mentioned above.

Particularly preferred peptides of the present invention include the following:

Designation	Portion of parent peptide
C-I-7	Ac-Cys(Acm)-(59-72)-OMe
C-I-6	Ac-(59-72)-OMe
C-I-5	Ac-(60-72)-OMe
C-I-4	Ac-(61-72)-OMe
C-I-3	Ac-(62-72)-OMe
C-I-2	Ac-(63-72)-OMe
C-I-1	Ac-(64-72)-OMe

wherein Ac represents acetyl;
OMe represents methoxy; and
Acm represents acetamidomethyl.

Furthermore, Leu-amide or ester may be advantageous at position 15 of peptide C-I-7 (equivalent to position 72 of IL-2). Alternatively, a D-Leu at position 2 of C-I-7 or indeed any D-amino acid at position 2 of any of the peptides may provide the advantage of increased half-life *in vivo* by preventing the actions of aminopeptidases. Moreover, introduction of a D-amino acid at any position in the peptide may help confer resistance to peptidases. For example, in C-I-6 one of the sensitive sites has already been localised at the bond between Leu(66)-Glu(67). Furthermore, all the above peptides are protected at both the amino and carboxy terminal ends, which also confers resistance to enzymatic degradation.

A series of peptides derived from the primary sequence of IL-2 has been shown to exhibit biological activity. This activity has been shown in a number of models.

Although the peptides of the invention do have some IL-2 like properties, they do not mimic all of the actions of IL-2. Thus, when peptides of the invention are tested in the CTLL assay (Robb, R.J. in *Methods in Enzymology*, **165**, Eds G. diSabato, J.J. Langone, H. van Vunakis, Academic Press Inc, Orlando pp 493-525, 1985), which is classically used to detect IL-2 activity, it does not induce the proliferation characteristic of IL-2 in these cells.

However, if peripheral blood mononuclear cells are prestimulated with the lectin phytohemagglutinin, the peptides of the invention, like IL-2, are able further to enhance proliferation when added to cultures 24 h later. Furthermore, in the CTLL assay mentioned above, if target cells are preincubated with a peptide of the invention before exposure to IL-2 or if the peptide is maintained during exposure to IL-2 then the apparent potency of the IL-2 is increased, i.e. half maximal stimulation of the cells is achieved with a lower dose of IL-2.

The peptides of the present invention are potentially useful in a wide variety of areas. One property of the peptides is their effect on the activation of macrophages. A number of investigators have described the so-called growth stimulating phase of macrophages which is distinguished from the (tumour cell) cytotoxicity phase (Okulov *et al*, *J. Cancer Res. Clin. Oncol.*, **118**, 537-541, 1992). It has now been found that the peptides of the present invention can act on

activated macrophages which are expressing heightened cytotoxic properties to switch them into a growth stimulating mode.

This action has been found to be dependent on the preactivation of the macrophages. Incubation of resting macrophages with the peptides of the present invention gives only the weakest of reactions.

In addition to their action on activated macrophages, the peptides of the present invention also have the ability to enhance the processes of tissue regeneration and reparation.

In a skin wound healing model, the peptides significantly enhanced wound healing of permanently disturbed skin wounds when applied either topically or intraperitoneally.

Thirdly, in a series of studies described in the examples below, the peptides have been found to be effective in promoting restoration of liver function. It has been shown that the peptides are capable of restoring the protein synthesising function of the liver and significantly inhibiting carbon tetrachloride induced elevation of aspartate and alanine-aminotransferases, γ-glutamyl transpeptidase and alkaline phosphatase.

The peptides of the invention have, moreover, been found to have immunosuppressant properties and, in particular, it has been demonstrated that they are capable of significantly reducing delayed type hypersensitivity reactions.

Clearly, therefore, the peptides of the present invention have useful biological properties. Moreover, they do not simply mimic the action of IL-2 but, rather, enhance certain useful activities whilst being free of the majority of the toxic side effects associated with IL-2. The inflammatory properties associated with IL-2 seem to be absent in many cases and under some circumstances it seems that the peptides can act as anti-inflammatory agents.

Therefore the peptides of the present invention are likely to be of therapeutic use in a method for the treatment of a number of diseases and conditions, the method comprising administering to a patient an effective amount of a peptide according to the invention. Broadly speaking the general type of disease or illness in which the invention will be useful includes any condition in which IL-2 may be beneficial although, because the peptides of the present invention fail to exhibit some of the toxic effects of IL-2 (for example the inflammatory effects), the peptides may occasionally be useful for conditions where IL-2 would not be expected to be beneficial. In particular, the peptides are expected to be useful in the treatment of cancers. In addition, the ability in some circumstances to transform macrophages into a "stimulatory" mode may manifest as an antiinflammatory activity. Finally, all circumstances where tissue regeneration and repair is of importance should be improved by the peptides.

In a second aspect of the invention there is provided the use of a peptide of not more than 50 amino acids in length comprising a fragment of at least 5 amino acids taken from the region extending from amino acid residues

58 to 72 of IL-2 and having the sequence:

58 59 60 61 62 63 64 65 66 67 68 69 70 71 72
Cys-Leu-Glu-Glu-Glu-Leu-Lys-Pro-Leu-Glu-Glu-Val-Leu-Asn-Leu;

a fragment substantially homologous thereto or a derivative of such a fragment in the preparation of an agent for the treatment of diseases and conditions in which IL-2 may be beneficial.

The type of diseases and conditions in which the peptides of the present invention may be beneficial include the following:

viral, bacterial or chemically induced hepatitis and meningitis;
rheumatoid arthritis;
sepsis/septic shock;
inflammation of the skin and superficial tissue resulting from infections, contact with irritant or allergic substances;
ARDS (adult respiratory distress syndrome);
in organ or bone marrow transplant patients;
cancer (optionally in combination with other treatments such as chemotherapy and/or radiation);
wound, tissue and organ healing;
recovery from toxicity induced by consumption of alcohol, medication and drugs of abuse;
recovery from surgical trauma;
Crohn's disease;
ulcerative colitis;
inflammatory bowel disease;
auto-immune disease;
multiple sclerosis;

AIDS;
inflammation of the muscles and joints;
regeneration of peripheral nerves; and
restoration of functional activity of liver enzymes.

In addition, the peptides can be used to increase the immune response to an antigen, thereby acting as an adjuvant and increasing the production of antibodies either as a vaccine, or for experimental purposes.

In order to treat these diseases and conditions the peptides may either be administered alone or in combination with other agents for example known antibacterial, antifungal or antiviral agents or steroidal or non steroidal anti inflammatory agents. When used in the treatment of diseases such as cancer, the peptides may be used in combination with other anti-cancer therapy including chemotoxic agents and immunomodulators such as α- or γ-interferon, IL-2 or other IL-2 derivatives or TNF-α and, when used in the treatment of transplant patients, other agents will preferably be used in combination with the peptides.

The preferred peptides and derivatives are as described above in relation to the first aspect of the invention.

The linear peptides described in this invention may be prepared by any process, such as conventional solid phase peptide synthetic techniques. (see "Solid Phase Peptide Synthetic Techniques", 2nd ed., J.11. Stewart, J.D. Young, Pierce Chemical Company, 1984, ISBN: 0-9350,40-03-0). Another possibility is solution phase techniques.

Cyclic forms of these peptides may be prepared by known techniques, such as, for example, described in Y. Hamada, *Tetrahedron Letters*, **26**, 5155-5158 (1985). The cyclic peptides may be established in the form of an S-S bridge between two Cys-residues and/or reacting the carboxy terminal amino acid residue with the amino terminal residue and/or reacting the amino terminal residue with for example the gamma carboxyl group of Glu.

The peptides will generally be included in a pharmaceutical or veterinary formulation and, therefore, in a third aspect of the invention, there is provided a pharmaceutical or veterinary composition comprising a peptide of not more than 50 amino acids in length and comprising a fragment of at least 5 amino acids taken from the region extending from amino acid residues 58 to 72 of IL-2 and having the sequence:

58	59	60	61	62	63	64	65	66	67	68	69	70	71	72
Cys	Leu	Glu	Glu	Glu	Leu	Lys	Pro	Leu	Glu	Glu	Val	Leu	Asn	Leu;

a fragment substantially homologous thereto or a derivative of such a fragment together with a pharmaceutically and/or veterinarily acceptable carrier.

The compositions may also include other components, for example any known antibacterial and/or antiviral agent and/or antifungal agent or steroidal or non-steroidal anti-inflammatory agents.

The compositions of the present invention may be prepared simply by admixing the ingredients.

The compositions may be adapted for administration by the oral, parenteral, topical, nasal, buccal, rectal, or vaginal routes, or by inhalation spray or in other ways.

In particular, the peptides of the invention may be formulated for topical use, for inhalation by spray, for injection, for infusion or for oral administration and may be presented in unit-dose form in ampoules, tablets or in multidose containers with an added preservative. The compositions may take such forms as suspensions, solutions, emulsions in oily or aqueous vehicles or liposomal formulations, and may contain formulating agents such as suspending, stabilising and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g. sterile, pyrogen-free water, before use.

The compositions may contain from $1x10^{-6}$% to 99% of the active material. Other ingredients which may be included in the compositions include antimicrobial agents, preservatives and flavouring and/or colouring agents.

It is preferred that the compositions are administered in therapeutically or prophylactic effective doses. The dose administered daily will, however, be determined by the physician and will, of course, depend on the type and severity of the condition to be treated, for example, in the case of inflammation, on the degree of inflammation and inflammatory response. The size of dose will also, of course, depend on the size of the patient to be treated. However, in general, the dose will generally be in the region of 0.001-10000 mg of peptide per day, in particular 1-1000 mg per day.

The invention will now be further described with reference to the following non limiting examples and to the drawings in which:

FIGURE 1 is a histogram showing the effect of an IL-2 derived peptide of the invention on PHA-induced proliferation of peripheral blood mononuclear cells;

FIGURE 2 shows the potentiation of IL-2 activity in the CTLL assay by a peptide of the invention when the cells have been pre-incubated with the peptide;

FIGURE 3 shows the potentiation of IL-2 activity in the CTLL assay by a peptide of the invention when the cells have been co-incubated with the peptide;

FIGURE 4 shows the effect of peptides of the invention on induction of growth stimulating potential in macrophage supernates;

FIGURE 5 compares the effects of incubating resting macrophages with TPA or with a peptide of the present invention on tumour cell growth stimulation;

FIGURE 6 is a plot of the healing of full-thickness cutaneous wounds either untreated or treated with a peptide of the invention;

FIGURE 7 is a plot showing the action of various peptides of the invention on the liver function of mice after treatment with carbon tetrachloride; and

FIGURE 8 shows the action of a peptide of the invention on liver function of mice suffering

galactosamine induced toxicity.

Example 1

Effect of IL-2 derived peptides on mitogen-induced proliferation of peripheral blood mononuclear cells

MATERIALS AND METHOD

Blood samples were obtained by venipuncture or by leukopheresis from healthy volunteers after informed consent. 4-5 ml of blood was diluted 1:1 with Hanks' balanced salt solution (HBSS), layered on 2 ml of FICOLL™ density gradient solution, and peripheral blood mononuclear cells (PBMC) were separated by centrifugation at 1500 r.p.m. for 45 min. PBMC were collected from the liquid interface, washed 3 times in HBSS followed by centrifugation, and resuspended at 1 x 106 PBMC per ml in RPMI 1640 medium (Flow Labs, Scotland) supplemented with 10% foetal calf serum (BSSR, Minsk). For proliferation assay, PBMC were activated by incubation with phytohemagglutinin (PHA) (Sigma, 10 μg/ml) overnight, washed the next day and used for "IL-2 related activity assay".

IL-2 related activity assay

The assay was performed in 96-well U-bottomed tissue culture plates (Falcon, Oxnard, California, USA). In each well, 100 μl of PBMC (1 x 106 cells/ ml) were incubated with 100μl of peptide solution (at concentrations of 200 μg/ml, 20 μg/ml, 2 μg/ml and 0.2 μg/ml) or 100μl of recombinant interleukin 2 (IOS, Riga, Latvia, 10000 U/μg) at a concentration of 1000 U/ ml in complete RPMI 1640 medium. Control cultures had 100 μl of complete medium or 100 μl of PHA (10μl/ml). After 48h

incubation, each well received 10 µl of [3H]-thymidine (1 µCi/ml) and 2 h later the cells were harvested on paper filters, washed twice with cold 5% trichloroacetic acid and absolute alcohol, and air dried. [3H]-thymidine incorporation was determined by liquid scintillation counting (Delta Nuclear, Chicago USA). All tests were performed in at least triplicate.

Differences between treated groups and controls were estimated by Student's t-test. Level of significance was set at $p<0.05$.

RESULTS (Fig. 1)
As expected, IL-2 increased proliferation of PBMC over control values, and to a greater degree than was obtained simply by addition of further PHA (Note that all cultures, even "controls", had been exposed to PHA the day before performing the "IL-2 related activity assay"). Surprisingly, the peptide C-I-6, which comprises only a part of the sequence of IL-2, was also able to increase proliferation.

Example 2

Potentiation of IL-2 effect by pre-exposure of cells to C-I-6

METHODS
CTLL cells were incubated with C-I-6 at a range of concentrations for 90 min at 37°C and then washed three times to remove excess peptide. These pretreated cells were then plated at 104 cells/well in a range of dilutions of IL-2. After 24 h, 0.04 MBq of [3H]-thymidine was added to each well, and incorporation

of radioactivity over the subsequent 24 h was determined as a measure of proliferation.

RESULTS (Fig 2)

CTLL cells are dependent upon IL-2 for their growth, and the potency of a batch of IL-2 can be described as the amount (or dilution) necessary to cause 50% maximal growth of these cells. As shown in Fig 2, preincubation of cells in peptide C-I-6 resulted in half-maximal stimulation of cells at a lower concentration of IL-2, i.e. the apparent potency was increased.

Example 3

Potentiation of IL-2 effect by coincubation with C-I-6

METHODS

CTLL cells were incubated in a range of dilutions of IL-2, in the presence or absence of C-I-6 at a number of different concentrations. After 24 h incubation, treatment was as described in Example 2.

RESULTS (Fig. 3)

Coincubation with C-I-6 achieved half-maximal stimulation of CTLL growth with a lower concentration of IL-2. As discussed in Example 2, this indicates that C-I-6 increases the potency of IL-2 in this assay.

The results of Examples 1 to 3 demonstrate that some of the activity of the peptides of the invention is related to IL-2 activity. Thus, as described in Example 1, if peripheral blood mononuclear cells are prestimulated with the lectin phytohaemagglutinin, IL-2 is able further to

enhance proliferation when added to cultures 24 hours later and Peptide C-I-6 has a similar effect. Furthermore, as described in Examples 2 and 3, in the CTLL assay, if target cells are preincubated with C-I-6, before exposure to IL-2 or if C-I-6 is maintained during exposure to IL-2, then the apparent potency of the IL-2 is increased, i.e. half maximal stimulation of the cells is achieved with a lower dose of IL-2.

Example 4

The effect of IL-2 derived peptides on the production of tumour-cell growth inhibiting substances by macrophages

MATERIALS AND METHODS

Female C57B1/6 mice weighing 18-22g were obtained from the Rappoplovo animal breeding facility of the Academy of Medical Sciences, USSR.

Peritoneal macrophages isolation. Mice were killed by cervical dislocation. Peritoneal cells were collected by lavage with 5 ml of basal Eagle's medium (BEM, Institute of Poliomyelitis and Viral Encephalitis, Moscow), centrifuged at 200g for 10 min, and resuspended in the same medium at 1 x 106 cells/ml. 100μl of cell suspension was plated in each well of 96-well flat-bottomed plates (Nunc. Denmark) and incubated in 5% CO2, 95% air, 100% humidity at 37°C for 90 min. Non-adherent cells were removed by washing. 4-5 x 104 adherent cells remaining per well had a morphology typical of macrophages. For each experiment, a pool of peritoneal cells obtained from 3-4 mice was used.

Macrophages obtained as described above were cultivated in 200µl of complete medium (BEM supplemented with 10% heat-inactivated neonatal bovine serum) containing 2 mM L-Gln, 100 U/ml penicillin, 100 µg/ml streptomycin and, to achieve activation, well known activating agents such as lipopolysaccharide. At the point of maximal cytotoxicty, cells were further incubated in the IL-2 derived peptides to be tested: C-I-6 (1.0 µg/ ml) or C-I-1. C-I-2, C-I-3, C-I-4, C-I-5 or C-I-7 (10 µg/ ml). On the second day, cultures were washed and incubated for 18h in serum free medium. Supernatants were then aspirated and again tested in the tumour growth stimulation/inhibition assay.

Tumour cell growth stimulation/inhibition assay. P3X-63-Ag/8 mouse myeloma cells were maintained in complete medium and cultured under conventional conditions. For experiments, cells were seeded in 96-well plates (5 x 103 cells per well) in 100 µl of serum free medium. 100 µl of serum free macrophage culture supernatants were added to myeloma cells, which were cultured for 24h under conventional conditions. Cells were then pulsed with 1.0 µCi of [3H]-thymidine for 2h. Cells were harvested on filters and radioactivity was determined with a liquid scintillation counter Delta-300 (Nuclear Chicago).

Growth stimulation/inhibition was determined using the formula:

$$\frac{cpmE - cpmC}{cpmC} x100$$

and expressed as a % of control, where:

cpm C = counts per min in control wells (target cells incubated with supernatants obtained from unstimulated macrophages).

cpm E = counts per min in experimental wells (target cells incubated with supernatants obtained from macrophages having been in contact with IL-2 derived peptides).

All growth stimulation/inhibition experiments were performed in replicates of six wells.

RESULTS (Fig. 4)
In this assay, a large positive number indicates greater stimulation of growth of target myeloma cells. Of the peptides tested, C-I-4, C-I-5 and C-I-6 showed this effect, C-I-6 being the most effective (particularly considering it was tested at one-tenth the concentration of the other peptides).

Example 5

The effect of C-I-6 on the tumour cell growth stimulation/inhibition ability of resting macrophages

MATERIALS AND METHODS
Peritoneal macrophages obtained as described in Example 4 from SPF mice (breeding facility of the Central Institute of Microbiology and Epidemiology, Jena, DDR) were treated with TPA (1 nmol/ml) or C-I-6 (1 μg/ml) in complete culture medium under conventional conditions for 24 h. On days 1, 2, 3, 4, 5, 6 and 7, cultures were

washed and incubated for a further 18 h in serum free medium. These supernatants were aspirated and used for tumour growth stimulation/inhibition assay as described in Example 4.

RESULTS (Fig 5)

In this experiment, the kinetics of the response of resting macrophages (not stimulated with LPS) was investigated. Whereas TPA showed the strong biphasic response expected (Okulov *et al*, ibid), C-I-6, the peptide which was the most active in Example 4, caused only a slight, biologically insignificant change in the activity of macrophage supernates.

Examples 4 and 5 relate to the activation of macrophages by peptides of the present invention. A number of investigators have described the so-called growth stimulating phase of macrophages, which is distinguished from the (tumour cell) cytotoxicity phase (Okulov *et al*, *J. Cancer Res. Clin. Oncol.*, **118**, 537-541, 1992). Thus, as described in Example 4, activated macrophages which are expressing heightened cytotoxic properties can be switched into a growth-stimulating mode by incubation with a number of the peptides of the present invention. *In vitro*, this activity was detectable at 10 pg/ml and maximal at 1 μg/ml. When activation and its inhibition was performed *in vivo*, stimulation of growth promoting activity was detectable at 250 μg/kg and maximal at 2.5 mg/kg.

However, this property is greatly dependent on the preactivation of macrophages. Thus, when resting macrophages were incubated with peptides of the present invention, only the weakest of reactions (cytotoxicity

followed by growth stimulation, as described by Okulov *et al ibid*) was manifest. The chemical TPA, included as a positive control, showed the strong biphasic response expected.

Supernatants derived from stimulated macrophage cultures treated with peptides of the present invention contained no TNF-alpha/cachectin and low amounts of IL-1 indicating a reduction of inflammatory state and immune cell activation. As indicated above, both TNF-α and IL-1β are potent inflammatory mediators. Furthermore, the peptides differ from TGF-β, in that the latter inhibits both macrophage-mediated cytotoxicity and lymphocyte activity.

Example 6

The effect of C-I-6 on the healing of cutaneous wounds

METHODS

Mice were narcotised (80 mg of hexenal, i.p.) and full-thickness skin wounds were made with a round steel biopsy punch (9mm in diameter) on the depilated dorsal surface. On the 4th day the blood clot was removed. Blood clot removal was performed every day thereafter, until termination of the experiment.

C-I-6 was given every day i.p. or topically (in distilled water) at a dose of 2.5 mg/kg from day 1 to day 10 of the experiment.

The wound area was determined every other day by measuring with a micrometer.

TABLE 1 Effect of C-I-6 on healing of cutaneous wounds in the rat

Day	Control		C-I-6 i.p		C-I-6 topical	
	wound area	% of wounds healed	wound area	% of wounds healed	wound area	% of wounds healed
2	164±6.3	-	156.1±5.9	-	169.4±4.6	-
9	88.9±5.9	-	86.1±6.8	-	109.8±10.0	-
16	70.0±7.8	-	38.2±7.9*	7.7	42.4±9.5	-
23	38.3±10.0	-	15.0±5.8	36.4	13.8±6.4	27.3
30	21.1±7.4	27.2	5.1±2.1	72.7	0.4±0.4*	90.9*
37	7.5±5.6	54.4	0	100	0*	100*

* Statistically different from control, $p<0.01$

Results indicate that C-I-6 peptide enhances the rate of wound healing after either topical or intraperitineal administration.

Example 7

The effect of IL-2 derived peptides on liver function in tetrachloromethane poisoned mice

MATERIALS AND METHODS

Mice. Male HP mice weighing 18-22g were obtained from the Rappoplovo animal breeding facility of the Academy of Medical Sciences USSR. Peptides were administered daily i.p. in 0.2 ml of saline at a dose of 2.5 mg/kg/day for 3 days, starting 36 h after CCl_4 injection (7.5 ml/kg in olive oil (50% solution) s.c.). One day after the final injection of peptide, the duration of narcosis induced by thiopental was determined in intact, control (CCl_4

treated) and peptide (CCl_4 and peptide) treated mice.

RESULTS (Fig. 7)
The duration of narcosis of mice was increased from 7.5 minutes to 113.5 minutes following tetrachloromethane treatment indicating that the tetrachloromethane had caused damage to the liver.

Peptides C-I-3, C-I-4, C-I-5, C-I-6, C-I-7 caused a reduction in duration of narcosis. This indicates that they were able to facilitate recovery from liver damage previously induced by CCl_4.

Example 8

The effect of IL-2 derived peptides on liver function in mice with galactosamine-induced hepatitis

MATERIALS AND METHODS
Deficient liver function (as measured by delayed recovery from thiopental narcosis) was induced in mice by the administration of large amounts of galactosamine. Mice thus treated subsequently received C-I-6 (1.5 mg/kg i.p.) for four days, or control treatment.

RESULTS (Fig 8)
After induction of liver poisoning with galactosamine, the duration of thiopental narcosis time was approximately 60 min. Treatment with C-I-6 was able to reduce this to almost control levels.

This indicates that C-I-6 is able to cause recovery from liver damage previously induced by galactosamine administration.

Example 9

Comparison of C-I-6 effect to therapeutic effect of Prednisolone

MATERIALS AND METHODS

Deficient liver function, as measured by circulating levels of albumin and enzymes, was induced in rats by the administration of tetrachloromethane. Groups of rats then received no treatment (control) or treatment with C-I-6 (1.5 mg/kg) or Prednisolone for four days. At the end of this time, albumin and enzymes were measured. The results are shown in Table 2.

TABLE 2 Comparison of therapeutic effect of C-I-6 and Prednisolone

Treatment	Albumin g/dl	Alanyl amino-transferase U/l	Alkaline phosphatase U/l	Gamma glutamyl tansferase U/l
Intact rats	2.19 ± 0.05	29.6 ± 0.8	16.1 ± 3.6	19.8 ± 4.0
Control	1.82 ± 0.09*	48.5 ± 4.9*	38.8 ± 5.3*	55.1 ± 7.5*
C-I-6	2.21 ± 0.1**	36.6 ± 2.9**	20.4 ± 3.1**	24.8 ± 5.8*
Prednisolone	2.36 ± 0.08**	35.4 ± 2.8**	25.9 ± 5.6	37.3 ± 5.3

* $p<0.05$ in comparison with intact rats

** $p<0.05$ in comparison with control rats

These results indicate for several functional indicators, C-I-6 was more effective than Prednisolone at returning values to those obtained in intact rats without liver damage.

Example 10

The effect of C-I-6 on rat liver regeneration after partial hepatectomy

MATERIALS AND METHODS

The influence of C-I-6 on liver regeneration after partial (one third) hepatectomy in outbred white rats (Petrov Oncology Institute's animal breeding facility) was studied.

For three days before hepatectomy, rats (10 per each group) received C-I-6 treatment (at a dose of 2.5 mg/kg, i.p.), or control treatment. General histology, mitotic index and the index of labelled nuclei was assessed 24 and 48 h after the hepatectomy after pulse labelling in vivo with [3H]-thymidine. The results are shown in Table 3.

Table 3 Effect of C-I-6 on liver regeneration after hepatectomy

Time after hepatectomy(h)	C-I-6 treatment	Index of labelled nuclei (%)*	Mitotic (%)**
24	-	23.9 ± 3.6	21.1 ± 4.1
	+	13.2 ± 2.3	5.2 ± 0.9
48	-	10.2 ± 2.1	13.7 ± 3.4
	+	20.4 ± 4.5	33.7 ± 3.2

The experiment was carried out according to method of Higgins and Anderson 1931.

Values for both indices in non-hepatectomized rats was less than 2.0

* Counted from 3000 hepatocytes
** Counted from 1000 hepatocytes

The data indicate that initially, C-I-6 exerted some inhibition of proliferative processes in the regenerating liver. However, it later stimulated proliferation of hepatocytes significantly. Moreover, histological examination revealed more intensive cell destruction in the first 24 h of the experiment in C-I-6 treated livers. In addition, in treated animals substantially more glycogen granules were observed in hepatocytes, as comparison to controls.

The results of this experiment show that C-I-6 enhances regenerative processes in liver damage caused by physical trauma (hepatectomy) in addition to the toxic damage described in Examples 7 and 8.

As shown in Example 7, C-I-6 was found to be effective in promoting restoration of liver function. In a model of tetrachloromethane (CCl_4)-induced hepatitis, the duration of thiopental narcosis was determined after treatment with various IL-2 derived peptides. The duration of narcosis of mice was increased from 7.5 minutes to 113.5 minutes following tetrachloromethane treatment (7.5 ml/kg) indicating that the tetrachloromethane had caused damage to the liver. Peptide C-I-6, intraperitoneally administered at a dose of 2.5 mg/kg/day for 3 days after CCl_4 administration, caused a significant reduction in animal recovery time, indicating improved recovery of liver damage induced by CCl_4.

In Example 8, where hepatotoxicity was induced by galactosamine, C-I-6 was effective in inducing hepatic

recovery and consequently facilitating recovery from thiopental narcosis.

In mice given a sub-lethal dose of hepatotoxin, C-I-6 was able to reduce the incidence of mortality induced by CCl_4.

Further confirmation of the reparative effects of C-I-6 on hepatotoxicity was derived by determination of liver specific enzymes and protein synthesising function of the liver in Example 9. Liver-derived amino transferase levels in blood are considerably increased following liver pathology caused by acute viral hepatitis, chronic active hepatitis, alcoholism or other chemical or drug disturbances. In a series of experiments in mice and rats it was shown that peptide C-I-6 completely restores the protein-synthesising function of liver and significantly inhibits CCl_4-induced elevation of aspartate and alanine-aminotransferases, gamma-glutamyl transpeptidase and alkaline phosphatase. It was, moreover, shown that C-I-6 is more potent than Prednisolone in inducing the liver reparative process.

Hepatectomy is sometimes necessary following a number of diseased states; the ability of C-I-6 to induce liver regeneration was examined following partial hepatectomy in outbred rats in Example 10. In control animals the indices of mitotic activity of hepatocytes rose sharply after the first 24 hours, in contrast to this, C-I-6 treated animals suffered a delay in the increase in mitotic index after 24 hours. However, at 48 hours, C-I-6 treated animals demonstrated a pronounced increase in mitotic index. Thus, C-I-6 is able to enhance the regeneration and repair of liver tissue.

Example 11

Investigation of the low toxicity of C-I-6.

Adult rats received intraperitoneal injections of 1 or 10 mg/kg of C-I-6 daily for 7 days. At the end of this time, animals were sacrificed and examined for pathoanatomical abnormalities or changes in blood biochemistry.

The results are shown in Table 4.

Table 4. Biochemical and haematological characteristics after 7 days administration of C-I-6.

	Treatment		
	Control	C-I-6 1 mg/kg	C-I-6 10 mg/kg
Protein (mg/ml)	88 ± 9	91 ± 11	90 ± 10
Urea (mM)	4.9 ± 1.4	6.0 ± 0.9	6.9 ± 3.1
Haemoglobin (mg/ml)	196 ± 16	195 ± 13	193 ± 16
Cholesterol (mM)	1.0 ± 0.7	0.7 ± 0.2	1.0 ± 0.3
Glucose (mg/ml)	0.54 ± 0.42	0.54 ± 0.30	0.84 ± 0.54
Ala-aminotransferase (U/l)	12.8 ± 2.4	15.2 ± 1.8	10.9 ± 3.1
Asp-aminotransferase (U/l)	20 ± 3.7	19.2 ± 2	18 ± 2.8
Erythrocytes	9.3 ± 1	9.1 ± 1.2	9.0 ± 0.8
Lymphocytes (%)	72 ± 7	77 ± 6	77 ± 8
Neutrophils (%)	169 ± 7	14 ± 7	17.9 ± 7

No significant differences between control and treated groups were seen.

Pathoanatomical findings also showed no difference between control and treated groups.

These experiments show that C-I-6 exhibits toxicity which is much lower than that of IL-2. As discussed above, IL-2 has serious toxic side effects which are not shared by C-I-6.

Example 12

Effect of C-I-6 on delayed type hypersensitivity (DTH) to tuberculin antigen.

METHODS

56 Balb/c mice were sensitised with live BCG vaccine (500 μg/mouse, intradermal injection). After 6 weeks, the tuberculin test was performed: Purified protein derivative (PPD) (900 units) was injected into one hind foot pad and saline into the contralateral foot pad. Simultaneously, C-I-6 (0.4, 2.0 or 10 mg/kg) was introduced i.p. 24 hours later, the DTH reaction was evaluated by measuring and comparing the thickness of foot pads of right and left legs. Groups of mice which received no C-I-6, or a mixture of amino acids were used as controls.

Three more groups of mice received C-I-6 24 hours after PPD injection. In this case, DTH reaction was evaluated 48 hours after PPD injection.

RESULTS

When injected at the same time as PPD, C-I-6 at doses of 2 and 10 mg/kg significantly reduced the DTH reaction. (Positive control mean = 0.35, C-I-6 mean = 0.18, $p<0.05$).

When injected 24 hours after PPD, none of the doses of C-I-6 had a statistically significant effect. Similarly, none of the controls (saline, amino acids) gave statistically significant changes in DTH.

DISCUSSION OF RESULTS

This example gives a clear indication that C-I-6 is able to reduce the development of immune reactions to substances to which an animal has become sensitised.

Example 13

Effect of C-I-6 on delayed type hypersensitivity to sheep red blood cells (SRBC).

METHODS

Mice: Outbred NMRI male mice, 2 months old, with a body weight of 25-29 g were used. The animals were maintained under SPF conditions, at 20±2°C, 45-55% RH, and food and water were provided *ad libitum*.

Test materials: C-I-6 was dissolved in Dulbecco's balanced salt solution (DBSS). Cyclophosphamide was used as a positive control material.

Antigen: SRBC obtained by jugular puncture were stored as a 50% suspension at 4°C under sterile conditions before use. Immediately before use, 1 ml (for sensitisation) or 3 ml (for challenge) of 50% suspension were washed 3 times in saline and resuspended in saline.

Treatment schedule 1 - Effect on sensitisation stage: Animals were treated daily with intraperitoneal injections of C-I-6 (0.4, 2.0 or 10.0 mg/kg), cyclophosphamide (100 mg/kg) or the drug free vehicle (DBSS) on days -1, 0 and +1, where day 0 = day of sensitisation. Cyclophosphamide was given only on days 0 and +1 because of its extreme toxicity.

Treatment schedule 2 - Effect on challenge stage:

Animals were treated twice (20 hour interval) with intraperitoneal injections of C-I-6 (0.4, 2.0 or 10.0 mg/kg), dexamethasone (10 mg/kg) or drug free vehicle (DBSS) on days 5 and 6 after sensitisation. Challenge was on day 6, 4 hours after the last drug treatment.

DTH reaction: Mice (10 per group) were sensitised to SRBC by a single injection of 2 x 10^7 SRBC in ml saline (day 0). Five days later, mice were given 108 cells in 50 μl by injection into the left hind footpad. 50 μl injected into the left hind footpad served as control. 24 hours after the last injection mice were euthanised in CO2 and control and challenge footpads excised above the heel joint and weighed.

Results were expressed as:

$$\text{DTH index (\%)} = \frac{\text{Challenge paw weight - Control paw weight}}{\text{Control paw weight}} \times 100$$

Statistics: Student's t-test was used to evaluate the significance of data. $p<0.05$ was considered significant.

RESULTS

Table: Effects of C-I-6 on DTH reaction when given at sensitisation stage.

Group	Article	Dose (mg/kg)	DTH index % (sd)	% of control	p value
1	DBSS	-	13.95 (2.53)	100	-
2	Cyclophosphamide	0.4	4.88 (1.93)	35.0	<0.05
3	C-I-6	0.4	10.07 (0.86)	72.1	NS
4	C-I-6	2.0	8.17 (1.50)	58.5	NS
5	C-I-6	10.0	7.06 (0.69)	50.6	<0.05

Table: Effects of C-I-6 on DTH reaction when given at challenge stage.

Group	Article	Dose (mg/kg)	DTH index % (sd)	% of control	p value
1	DBSS	-	21.09 (1.91)	100	-
2	Dexamethasone	17.4	4.02 (0.59)	19.1	<0.001
3	C-I-6	0.4	13.62 (2.48)	64.6	<0.05
4	C-I-6	2.0	17.21 (1.49)	81.6	NS
5	C-I-6	10.0	19.15 (2.74)	90.8	NS

DISCUSSION OF RESULTS

Effect on sensitisation: At 10 mg/kg, C-I-6 caused a significant reduction in the DTH reaction. This reduction was less marked than that caused by the known immunosuppressant cyclophosphamide, but the latter agent was used at near toxic doses, whereas the C-I-6 showed no toxic effect.

Effect on challenge: The greatest reduction of DTH reaction at challenge was seen with the lowest dose of C-I-6 used, 0.4 mg/kg. Although this was statistically significant, it was far less effective than dexamethasone. Nevertheless, the latter drug is known to show undesirable side effects, as do most steroids, whereas C-I-6 appears to be devoid of toxic effects.

Clearly the peptides claimed in this invention have surprising biological properties. However, they do not simply mimic activities of the molecule from which they are derived (IL-2), although they do enhance certain activities of this molecule. They have been shown to have activity in animals, and thus can be expected to have therapeutic effect in man. In addition, acute toxicity evaluation of C-I-6 in mice revealed no adverse reactions at doses up to 25 mg/mouse, suggesting that toxicity would not be a limiting factor in the use of this peptide, unlike full-sized IL-2.

CLAIMS

1. A peptide of not more than 50 amino acids in length and comprising a fragment of at least 5 amino acids of the region extending from amino acid residues 58 to 72 of interleukin-2 (IL-2) and having the sequence:

58 59 60 61 62 63 64 65 66 67 68 69 70 71 72
Cys-Leu-Glu-Glu-Glu-Leu-Lys-Pro-Leu-Glu-Glu-Val-Leu-Asn-Leu;

a fragment substantially homologous thereto; or a derivative of such a fragment, for use in medicine.

2. A peptide as claimed in claim 1 which is linear for use in medicine.

3. A peptide as claimed in claim 1 which is cyclic for use in medicine.

4. A peptide as claimed in any one of claims 1 to 3 for use in medicine which has been derivatised by the conversion of the C-terminal carboxylic acid residue to a substituted or unsubstituted C_1-C_{20} alkyl amide, aryl amide, C_1-C_{20} alkyl ester, aryl ester, C_1-C_{20} alkyl ether or aryl ether.

5. A peptide as claimed in claim 4 for use in medicine, wherein the C-terminal carboxylic acid moiety has been converted to a methyl ester.

6. A peptide as claimed in any one of claims 1 to 5 for use in medicine, wherein one or more cysteine residues are substituted by C_1-C_{20} alkyl amide or substituted amide, triphenylmethyl, a C_1-C_{20} alkyl substituted carbamoyl group or a benzyl group.

7. A peptide as claimed in any one of claims 1 to 6 for use in medicine, wherein the nitrogen atom of the N-terminal amino acid is substituted with an aliphatic C_1-C_{20} acyl group or an aromatic acyl group.

8. A peptide as claimed in claim 7 for use in medicine, wherein the nitrogen atom of the N-terminal amino acid is substituted with a formyl, acetyl or propionyl group.

9. A peptide as claimed in any one of claims 1 to 8 for use in medicine and having one or more of the following substitutions:

one or more nitrogen atoms replaced by oxygen atoms such that the amine bonds are replaced by ester bonds;

conversion of hydroxy side chains to C_1-C_{20} ester or ether groups; or

substitution of one or more amino acids by a monomeric or polymeric carbohydrate or carbohydrate derivative.

10. A peptide as claimed in any one of claims 1 to 9, wherein one or more of the amino acids is in the D configuration.

11. Peptide C-I-7 (Ac-Cys(Acm)-(59-72)-OMe);
peptide C-I-6 (Ac-(59-72)-OMe);
peptide C-I-5 (Ac-(60-72)-OMe);
peptide C-I-4 (Ac-(61-72)-OMe);
peptide C-I-3 (Ac-(62-72)-OMe);
peptide C-I-2 (Ac-(63-72)-OMe);
peptide C-I-1 (Ac-(64-72)-OMe);

peptide C-I-7 with Leu-amide or ester at position 15;
peptide C-I-7 with D-Leu at position 2; or
peptide C-I-6 with D-Leu at position 9 and/or D-Glu at position 10;

wherein Ac represents acetyl, OMe represents methoxy and Acm represents acetamidomethyl, for use in medicine.

12. A tetra-, penta-, hexa-, hepta-, octa- nona-, deca-, undeca-, dodeca- trideca-, or tetradecamers of a peptide as claimed in any one of claims 1 to 11 for use in medicine.

13. The use of a peptide as defined in any one of claims 1 to 12 in the preparation of an agent for the treatment of diseases and conditions in which IL-2 may be beneficial.

14. The use as claimed in any one of claims 1 to 12 in the preparation of an agent for the treatment of:

viral, bacterial or chemically induced hepatitis and meningitis;
rheumatoid arthritis;
sepsis/septic shock;
inflammation of the skin and superficial tissue resulting from infections, contact with irritant or allergic substances;
ARDS (adult respiratory distress syndrome);
in organ or bone marrow transplant patients;
cancer (optionally in combination with other treatments such as chemotherapy and/or radiation);
wound, tissue and organ healing;
recovery from toxicity induced by consumption of alcohol, medication and drugs of abuse;

recovery from surgical trauma;
Crohn's disease;
ulcerative colitis;
inflammatory bowel disease;
auto-immune disease;
multiple sclerosis;
AIDS;
inflammation of the muscles and joints;
regeneration of peripheral nerves; or
restoration of functional activity of liver enzymes.

15. A pharmaceutical or veterinary composition comprising a peptide as defined in any one of claims 1 to 12, together with a pharmaceutically and/or veterinarily acceptable carrier.

16. The composition as claimed in claim 15, further including one or more of an antibacterial, antiviral, antifungal or steroidal or non-steroidal anti-inflammatory agent.

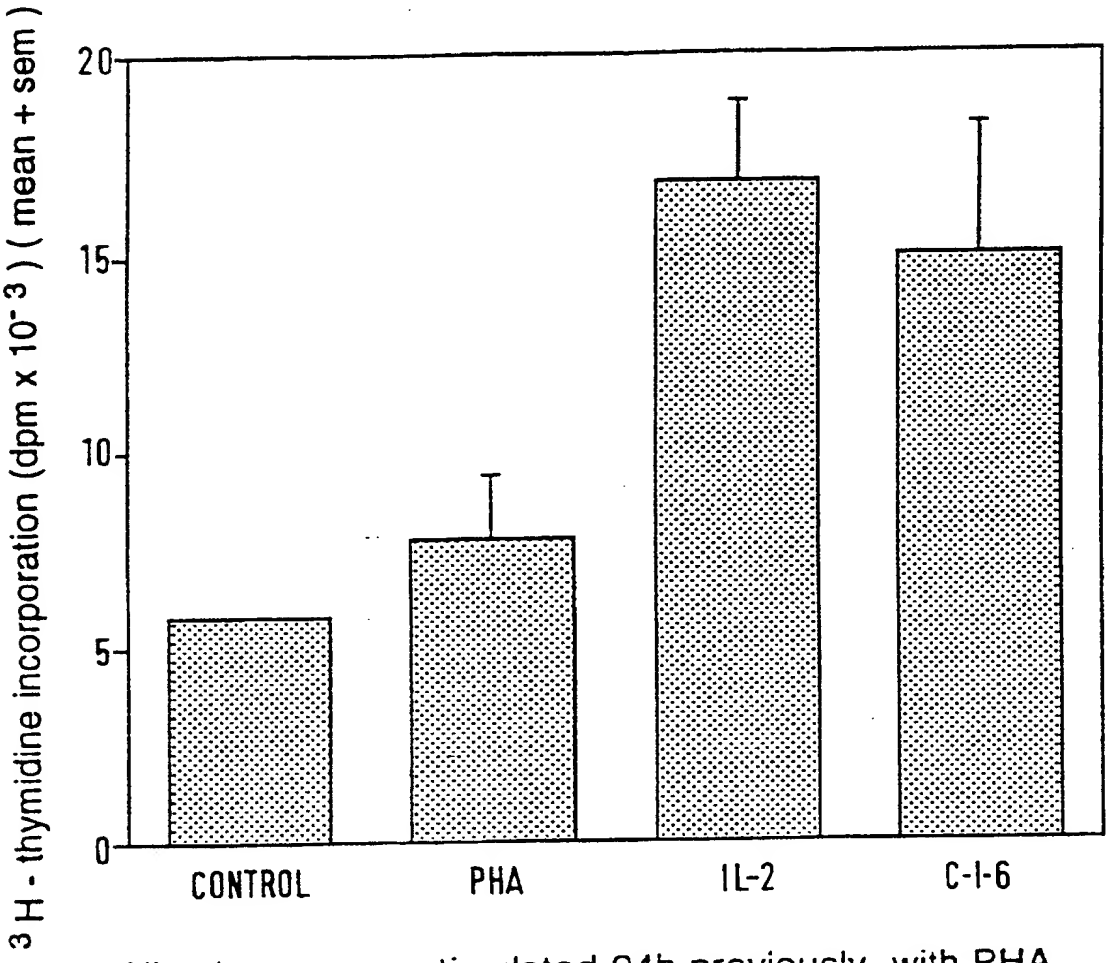
17. A method for the treatment of diseases and conditions in which IL-2 may be beneficial, the method comprising administering to a patient an effective amount of a peptide as defined in any one of claims 1 to 12.

18. A method as claimed in claim 17 for the treatment of:

viral, bacterial or chemically induced hepatitis and meningitis;
rheumatoid arthritis;
sepsis/septic shock;
inflammation of the skin and superficial tissue resulting from infections, contact with irritant or

allergic substances;
ARDS (adult respiratory distress syndrome);
in organ or bone marrow transplant patients;
cancer (optionally in combination with other treatments such as chemotherapy and/or radiation);
wound, tissue and organ healing;
recovery from toxicity induced by consumption of alcohol, medication and drugs of abuse;
recovery from surgical trauma;
Crohn's disease;
ulcerative colitis;
inflammatory bowel disease;
auto-immune disease;
multiple sclerosis;
AIDS;
inflammation of the muscles and joints;
regeneration of peripheral nerves; or
restoration of functional activity of liver enzymes.

EFFECT OF SYNTHETIC IL-2 FRAGMENTS ON PHA-INDUCED PROLIFERATION OF PERIPHERAL BLOOD MONONUCLEAR CELLS



All cultures were stimulated 24h previously with PHA

Control - No further treatment
PHA - Addition of further PHA
IL-2 - Addition of IL-2 (500 U/ml = 0.05µg/ml)
C-I-6 - Addition of C-I-6 (0.1µg/ml)

FIG.1.

POTENTIATION OF IL-2 ACTIVITY IN THE CTLL ASSAY BY C-I-6
(PREINCUBATION OF CELLS WITH C-I-6)

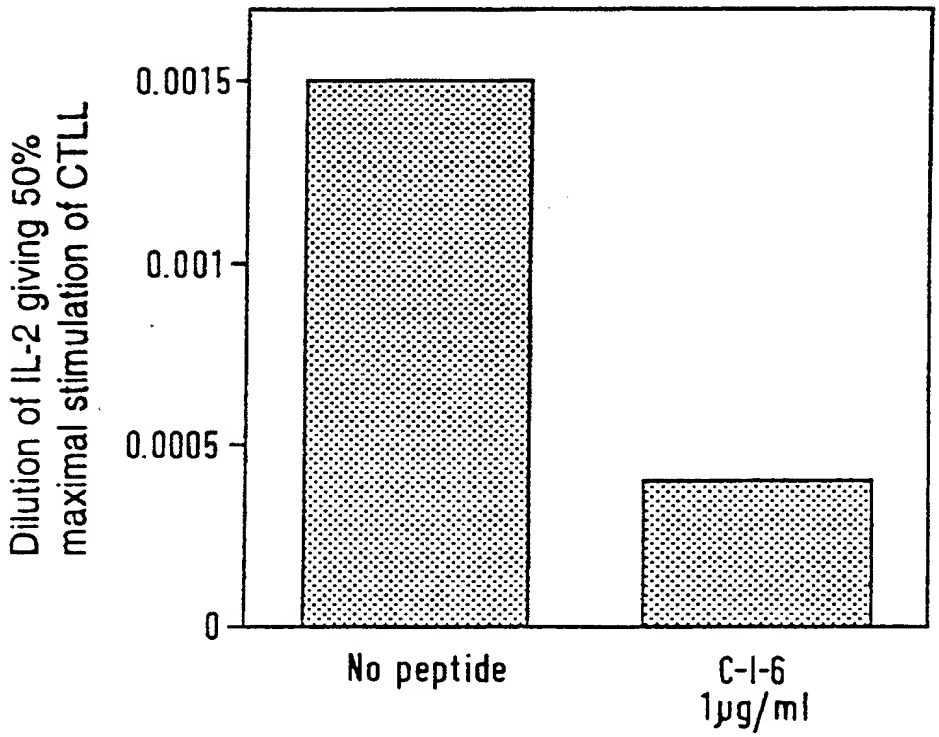


FIG. 2.

POTENTIATION OF IL-2 ACTIVITY IN THE CTLL ASSAY BY C-I-6
(CO-INCUBATION WITH C-I-6)

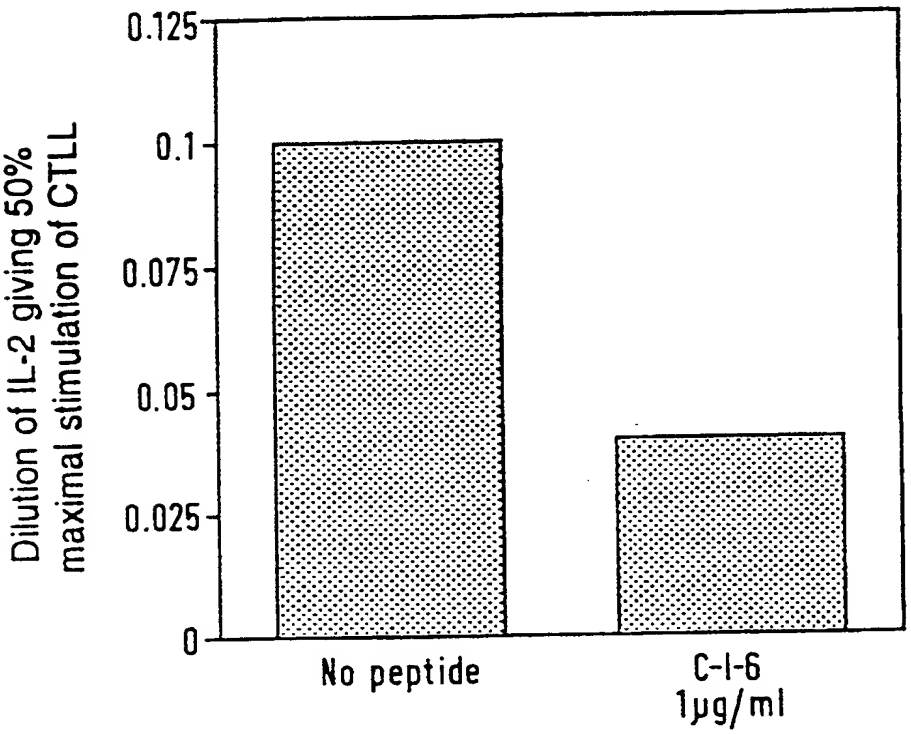
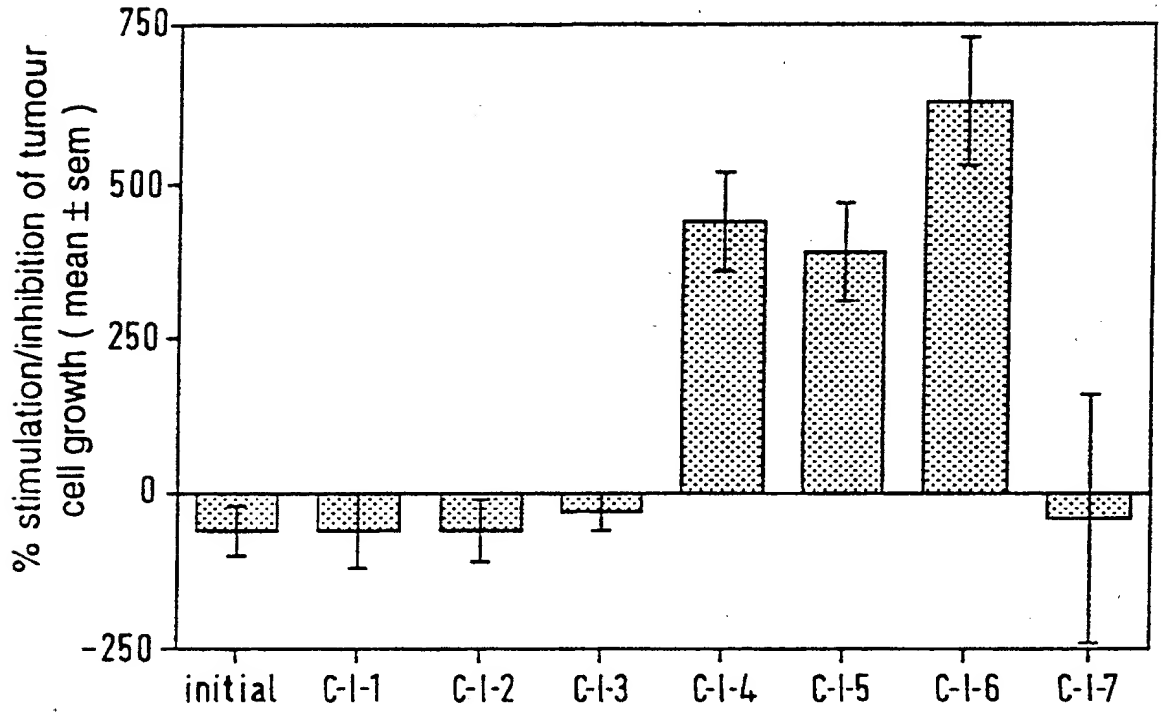


FIG.3.

EFFECT OF IL-2 RELATED PEPTIDES ON INDUCTION OF GROWTH STIMULATING/CYTOTOXIC POTENTIAL IN MACROPHAGE SUPERNATES



initial - Effect of supernate on tumour cells after incubation of macrophages with bestatin

C-I-1 etc - Effect after subsequent incubation with peptides.

FIG. 4.

EFFECT OF INCUBATING MACROPHAGES WITH TPA OR C-I-6 ON TUMOUR CELL GROWTH STIMULATING/CYTOTOXIC POTENTIAL

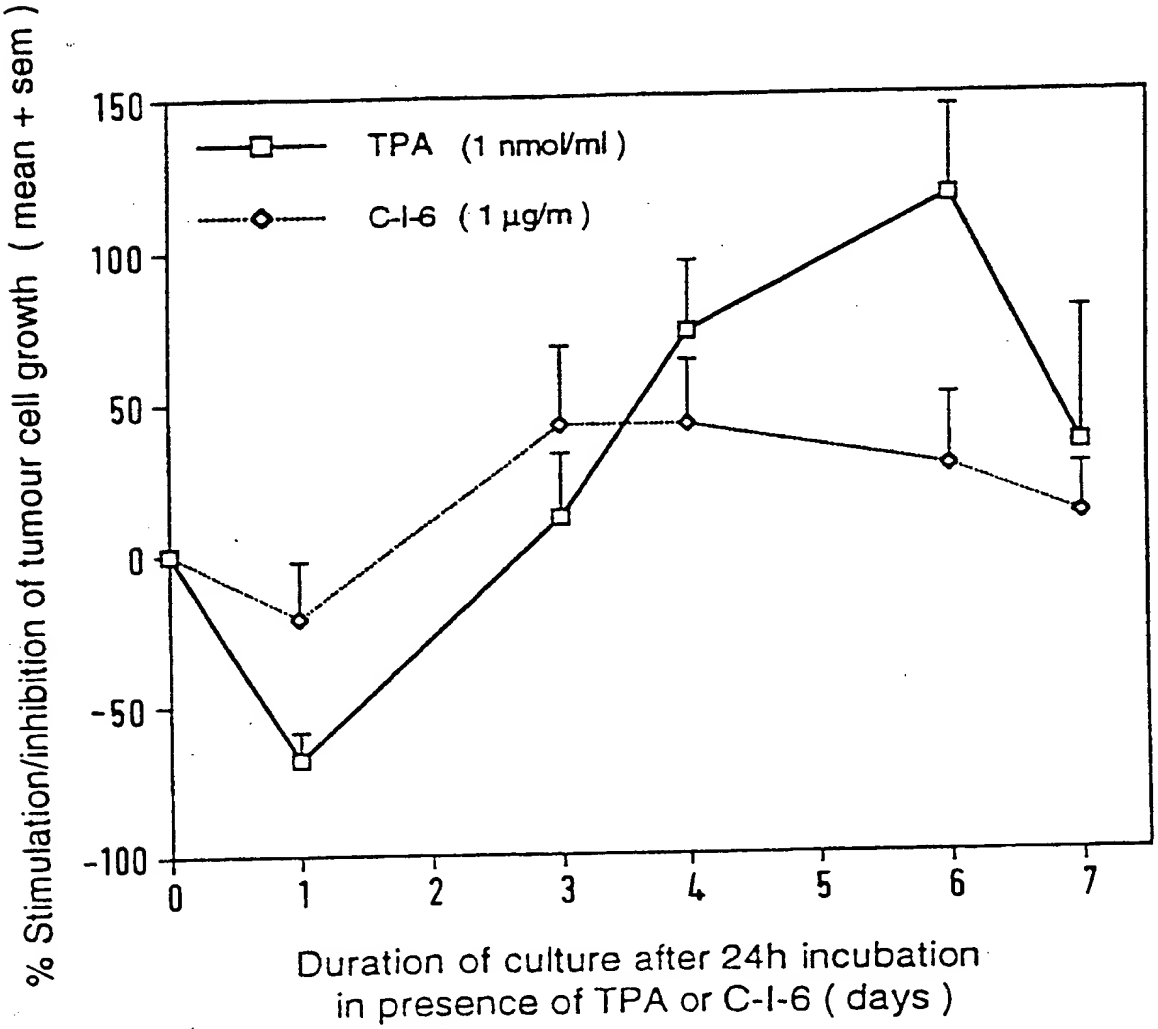


FIG.5.

HEALING OF FULL-THICKNESS CUTANEOUS WOUNDS

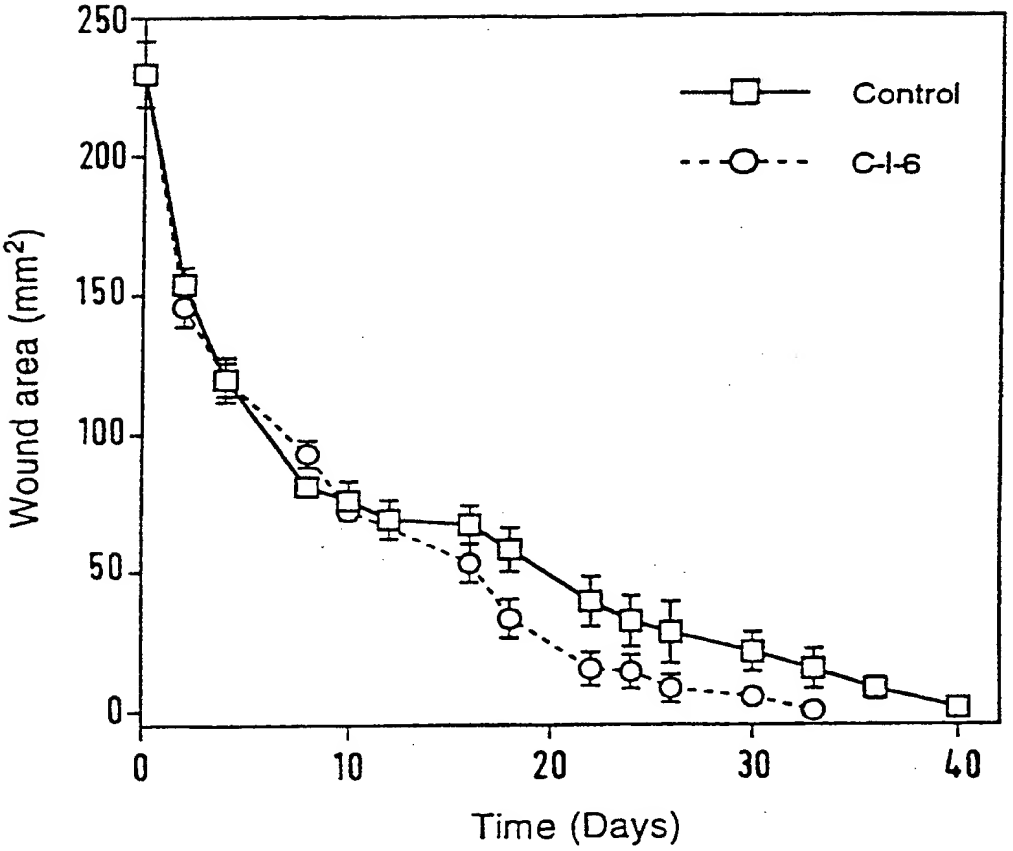
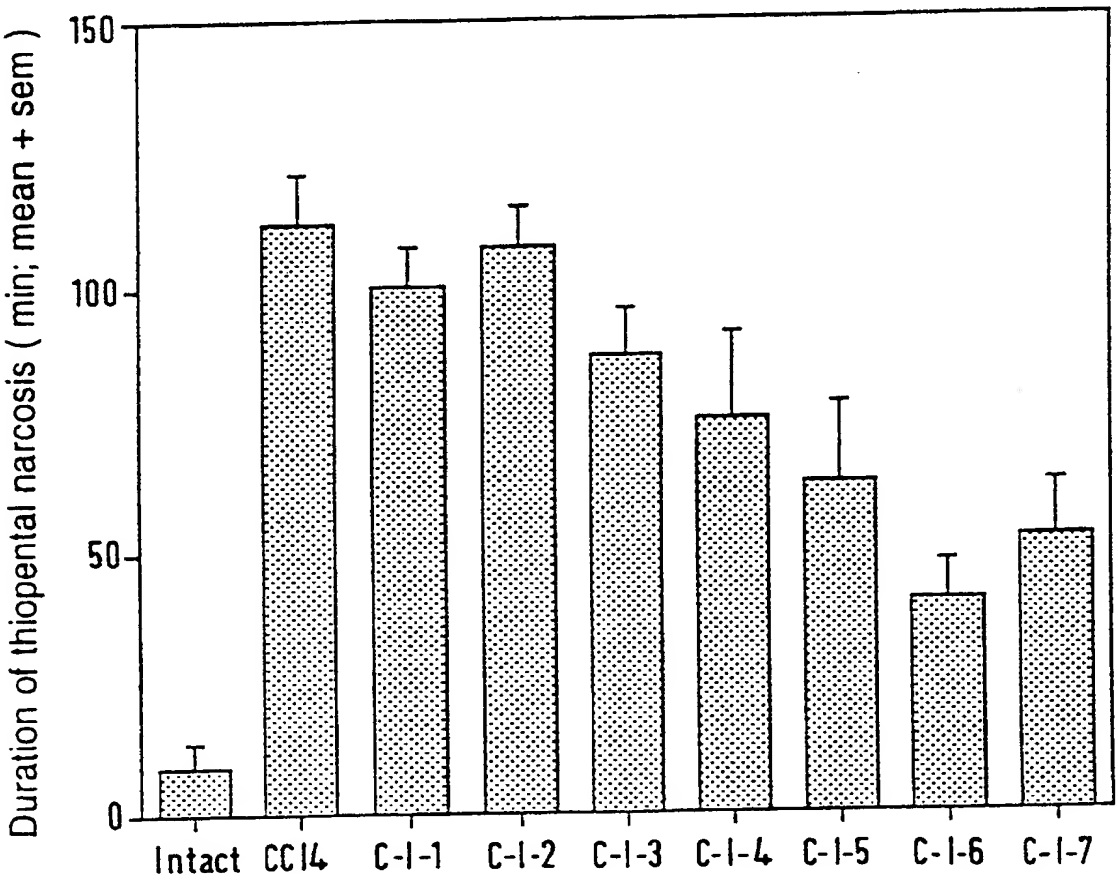


FIG.6.

ACTION OF SEVERAL IL-2 RELATED FRAGMENTS ON LIVER FUNCTION OF MICE AFTER CCl_4 TREATMENT



Intact - Untreated mice

CCl_4 - CCl_4 Treated mice

C-I-1 etc. - Mice treated with CCl_4 and peptide indicated

FIG. 7.

ACTION OF C-I-6 ON LIVER FUNCTION OF MICE SUFFERING GALACTOSAMINE-INDUCED TOXICITY

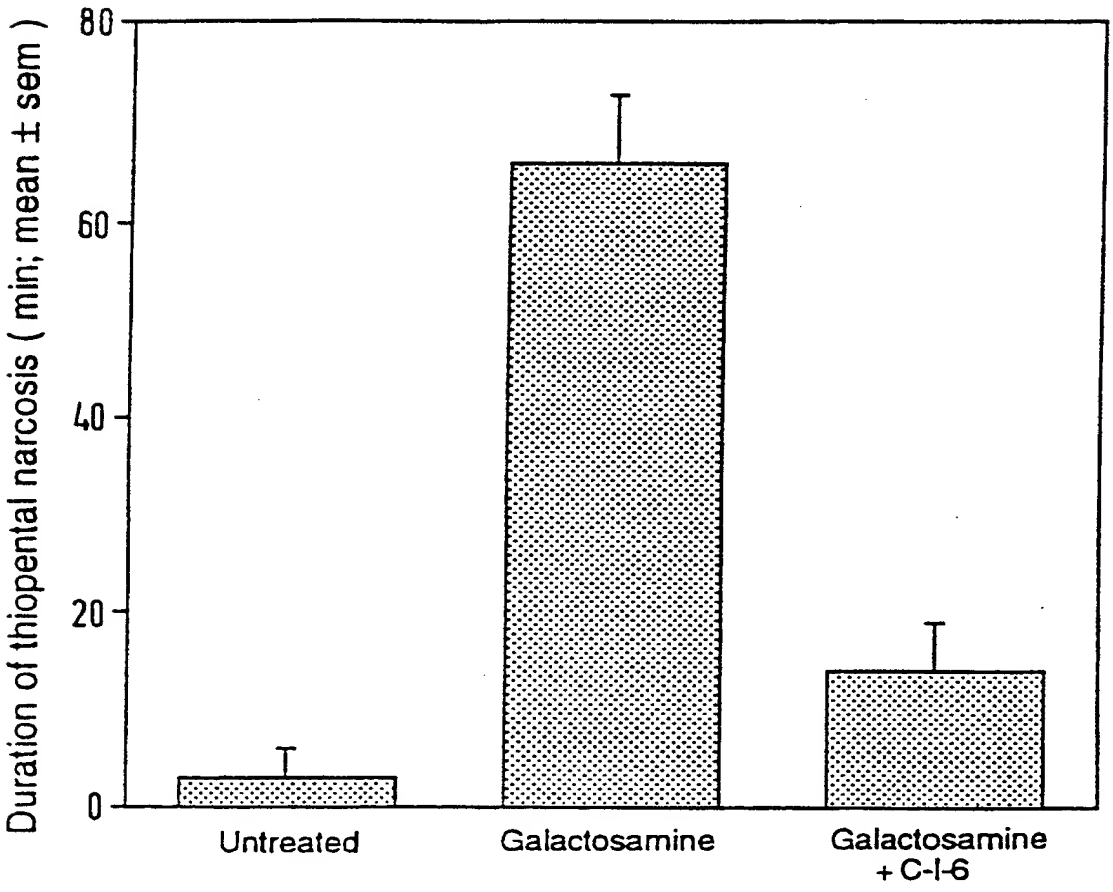


FIG.8.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 94/01349

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C07K7/00 A61K37/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,91 06562 (UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL) 16 May 1991 see claims 4,8-10 ---	1,2
X	WO,A,89 03221 (GENELABS, INCORPORATED) 20 April 1989 see page 10 - page 11; table 1 see claims ---	1,2
A	BIOORGANITCHESKAJA CHIMIA, vol.19, no.1, January 1993 pages 21 - 32 T. BALASHOVA ET AL 'Conformation of the complex of human interleukin-2 fragment (66-72) with the anti-interleukin-2 monoclonal antibody' see abstract --- -/--	1,2,11

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 September 1994	27.10.94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Le Cornec, N

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BIOORGANITCHESKAJA CHIMIA, vol.15, no.7, 1989 pages 908 - 921 L.V. ONOPRIENKO ET AL 'Synthesis and immunogenic properties of peptides corresponding to 59-72 and 25-36 sequences of human iL-2' see abstract ---	1,11
A	EP,A,0 157 643 (SCRIPPS CLINIC AND REARCH FOUNDATION) 9 October 1985 * see the whole document especially figure 1, the claims, table 1 * ---	1,2,11
A	VOPROSY ONKOLOGII, vol.37, no.7-8, 1991 pages 826 - 830 A.G. USHMOROV ET AL 'The effect of a synthetic fragment of human interleukin-2 on the experimental growth and angiogenesis of sarcoma 180 in mice' see abstract ---	1,2,11, 13,15
A	BIOORGANITCHESKAJA CHIMIA, vol.17, no.11, 1991 pages 1470 - 1486 T.A. BALASHOVA ET AL 'Proton NMR study of the complexes formed by monoclonal antibody against human interleukin-2 and its Fab-fragment with synthetic peptides,the fragments of interleukin-2' see figure page 1472 see abstract -----	1,2,11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB94/01349

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 17 and 18 are directed to a method of treatment of (diagnostic method practised on) the human/animal body (rule 39.1(iv)PCT) the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

information on patent family members

International application No.
PCT/GB 94/01349

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9106562	16-05-91	NONE		
WO-A-8903221	20-04-89	AU-A-	2784789	02-05-89
EP-A-0157643	09-10-85	US-A-	4636463	13-01-87
		AU-A-	4119085	01-11-85
		JP-T-	61501912	04-09-86
		WO-A-	8504653	24-10-85